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AN ACCOUNT
OF
SOME RESEARCHES INTO THE NATURE AND ACTION OF SNAKE VENOM.

By Captain ROBERT HENRY ELLIOT,
M.B., B.S.Lond., F.R.C.S.Eng., D.P.H.Camb.,
Indian Medical Service, Madras.

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I.—PRELIMINARY REMARKS ON THE METHODS EMPLOYED.

Method of Collecting and Storing Venom, Bile, etc.

In order to collect and store venom, bile, etc., the snake is first chloroformed and nailed on to a deal table; the blood is then collected by manipulations to be described later on, and the gall bladder is removed by making a long median ventral incision over it, cutting out the bladder together with the surrounding fat, and then making an incision into the most dependent part of the sac with a sharp pair of scissors; the bile flows into an evaporating dish, which is at once removed to a water bath kept at a temperature of 100° F.; as soon as the contents of this dish have dried the vessel is placed under the receiver of an air-pump, over concentrated sulphuric acid, and the air is exhausted; after twelve hours in the receiver the dish is removed, and the now dry bile is easily separated from the bottom of the dish, with the aid of a spatula or knife; it is at once placed in a dry glass tube, and tightly corked till required for use.

After removing the gall bladder the poison sacs are next dissected out, and their contents squeezed into a dry watch-glass, which is placed in a cupboard for a few hours till the poison dries into scales; if the atmosphere is damp, as is the case in the monsoon, the venom is dried by floating the watch-glass on a water bath at 100° F. for a couple of hours. The ease with which the poison separates from the glass when dry is remarkable; tapping the bottom of the glass is enough to separate the whole amount of venom from it in a few seconds. The dry poison is removed without delay into glass tubes previously dried at a high temperature, and allowed to cool before use; the tube is tightly corked as soon as filled. When undertaking a series, or a number of series of experi-

ments, the total stock of poison which it is intended to use is transferred to a glass mortar, finely pounded, and intimately mixed; it is then stored as before in a well-corked bottle and kept in the dark.

Every manipulation is carried on with the strictest attention to the prevention of the introduction of septic matter, all vessels being sterilised by heat beforehand, carbolic lotion 1-20 being used for such instruments as cannot be safely made hot; all corks used undergo a prolonged baking on a tin plate above the flame of a spirit lamp.

I have had frequent occasion to notice the correctness of a statement, made to me by Professor Fraser, that the poison of the Russel viper dries in long needle-shaped crystalline-looking masses unlike the shorter fracture of the dried poison of the cobra.

While working with live snakes a tourniquet of india-rubber tubing, a sharp knife, a cautery of some handy form, and a stock of crystals of potassium permanganate should always be kept handy. It is well, when possible, to have another medical man present; accidents occur when least expected, and the means to combat them should be ready at moment's notice. I should be wanting in gratitude if I failed here to acknowledge the debt I owe to Captain Samman, R.A.M.C., whose prompt action saved me from the very unpleasant consequences which might have followed a serious accident with which I met whilst handling a Russel viper. Captain Samman at once applied a firm ligature, and followed the fang puncture down to the bone with a knife: he then sucked the wound dry, and finally filled it with crystals of potassium permanganate. The result was that I escaped with nothing more than a painful sloughing wound. In the future a stock of Calmette's antivenene will always be kept at hand.

Standardisation of Solution and Mode of Administration.

When it is intended to perform a series of experiments, a rough calculation is first made of the total quantity of poison likely to be required, and a quantity in excess of this is carefully weighed out in decimals of a gram, on a scale showing $\frac{1}{10}$ mg. In order to minimise any possible error in weighing the writer never weighs out less than 20 mg. of poison at a time; each milligramme of venom is then dissolved in 1 or in 10 c.cm. of freshly boiled and cooled water, according to the strength of solution required. It will be noticed that these two solutions are respectively of the strength of 0.001 gram and 0.0001 gram of venom per c.cm. of water; the weaker solution is only used when the doses to be given are so small as to render their calculation difficult otherwise.

While conducting a series of experiments the solution is frequently stirred with a glass rod in order to ensure the evenness of the strength of solution. For each dose some solution is drawn up into a 10 c.cm. syringe, and the air having been expelled, the exact amount which it is required to inject is marked off by the aid of the revolving button on the piston axis; the dose is then given, either subcutaneously, or otherwise as may be desired. The syringe is sterilised by filling it several times in succession before operation from a beaker of rapidly boiling water.

Parallel series of experiments were conducted to ascertain whether the degree of dilution of the venom in the above-

named two strengths influenced the lethal properties of the dose. The length of this article forbids the publication of these tables here; but the result showed most clearly that the influence of the 10-fold dilution was inappreciable. It will be noticed, however, that in the various series of experiments one or other strength has been rigidly adhered to.

Preparation of Animal.

The animal is prepared for the injection by cutting off fur or feathers, and carefully washing the skin with 1-20 carbolic lotion.

Before undertaking any series of experiments it is necessary that all preliminaries should be carefully arranged beforehand in order to avoid confusion. Each animal of the series is therefore first weighed in pounds, ounces, and quarter-ounces; the result is converted into kilogrammes.

Means for subsequent identification are next taken; rabbits are branded in one ear with a serial number, care being taken to brand deep enough to destroy the fur and fur-bearing skin and no more; if less is done the mark wears out, while if more is done the result is sloughing, cicatrisation, and confusion; the animals scarcely seem to feel the branding. Fowls are marked by means of a wooden tally tied on under one wing. In all cases the colour, sex, etc., of the animal is noted.

The weight in kilos. being known, and the dose per kilo. having been decided on, a simple multiplication gives us the total dose the animal is to be given; and knowing the strength of our solution of venom, another easy calculation gives us the actual dose required in cubic centimetres and fractions of a centimetre.

The following columns in the experiment book can now be entered:

Serial No. of Experiment.	Date.	Hour.	Identifying Marks.	Weight in Kilos.	Dose per Kilo.	Actual Dose in c.cm.	Remarks.	Course of Events.

The first seven columns should be entered before starting to work on the series, the others subsequently.

The preparation of the mongoose requires a separate paragraph. These animals are so vicious, so wonderfully agile in their movements, and so strong and active, that it is advisable to weigh them, calculate their dose of poison, and administer it at one and the same time; they were brought in cages by the men of a caste who spend their lives in such occupations; in order to catch them, a running noose was passed through a hole in an iron rod, and this was insinuated over the animal's neck through the slightly opened door of the cage, the noose was then quickly pulled tight, and the animal was dragged out, securely tied, weighed and dealt with at once. In spite of every precaution, both I and one of my assistants received severe bites; these were delivered down to the bone in the fraction of a second, and were extremely painful.

Calculation of Dose: Idiosyncrasy of Animals.

Before starting to work on any species of animal, a long series of experiments was first undertaken to ascertain the dose of the poison to be used which would be certainly fatal in a limited period of time. At first an effort was made to work with the minimum lethal dose per kilo., but it was soon

found that this method introduced many difficulties. It was found, for example, that a dose of cobra venom corresponding to 0.0004 gram was the lowest dose per kilo. fatal to a rabbit; on the other hand, higher doses than this from time to time proved non-lethal, and rabbits occasionally recovered from doses as high as 0.0007 per kilo., though this was very rare.

If one fact stood out in bolder relief than any other it was this: that the personal element (if one may be excused the term applied to animals) is a factor never to be forgotten. Granted that the greatest care is taken to weigh the animals under the same conditions, that the poison is from the same stock, and most carefully weighed and apportioned, that every effort is made to inject the venom into the same tissues in all experiments, and to keep all known conditions as even as possible, granted all this, there still remains an element of uncertainty as to the fate of the subject which I can only attribute to "the personal factor." In order to eliminate this factor as far as possible, the dose chosen has always been one capable of certainly causing death, or at least of producing serious symptoms of snake poisoning.

In order to impose a further check on results, no series of experiments was undertaken without putting aside one or more animals as control snake-poison subjects. By this means the standardising of the lethal dose was kept as accurate as possible. This was the more necessary, because it was noticed that certain batches of animals presented a greater resistance than others, possibly due to the method of feeding, etc.

Lethal Dose for Various Animals.

Rabbits will often appear to get over the first effects of the venom, and will look as if on the way to recovery, but loss of weight continues, and after a longer or shorter period animals will succumb which at first sight one hoped would survive; their lower resistance to these secondary effects of venom poisoning renders them very inferior to fowls as subjects; the latter make a better fight to begin with, and do not suffer to anything like the same extent from remote sequelæ. Rabbits in the hills of India are also very susceptible to the influences of the frequent and trying changes of climate which prevail.

Rabbits.

All doses of cobra venom below, and including 0.0003 gram per kilo. of body weight proved non-lethal; doses of 0.0004, 0.0005, and 0.0006 gram per kilo. were lethal in most cases, but could not be certainly relied on; even 0.0007 gram per kilo. occasioually failed to kill, but it never failed to produee grave and well-marked signs of cobraism; doses of 0.0008 and 0.0009 were fatal in a very few hours. A dose of 0.0007 gram per kilo. killed in from six to eighteen hours as a rule, but life might be prolonged for days; it was accordingly seleeted as the dose for most of the experimcnts. The above conelusions appear to harmonise well with Professor Calmette's observations on dosage in his lecture delivered in the laboratories of the Royal Colleges on July 27th, 1896.

Daboia venom iu doses of 0.0004 up to 0.0009 took a fortnight or more to kill; even so high a dose as 0.001 took twelve days to kill, and one of 0.002 took seven days; a dose of 0.003, however, killed with very fair uniformity in under twelve hours; 0.004 killed in four hours and a-half. The dose of 0.003 gram per kilo. was, therefore, ehosen for daboia poison experiments.

Fowls.

Cobra venom in doses up to and under 0.001 proved non-lethal; 0.0015 gram per kilo. killed in fifty hours; 0 002 and 0.0025 killed in over twelve hours, and 0.003 in between six and twelve hours; these latter doses were therefore selected.

Daboia venom in doses of and above 0.003 gram per kilo. of body weight proved fatal; 0.003 gram killed in about twelve hours; all higher doses also proved lethal.